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TECHNICAL MANUSCRIPT 556

A PLAQUE ASSAY FOR <u>RICKETTSIA</u> <u>TSUTSUGAMUSHI</u>

Joseph E. McDade Peter J. Gerone

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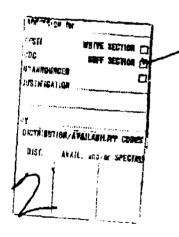
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A PLAQUE ASSAY FOR RICKETTSIA TSUTSUGAMUSHI

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Peter J. Gerone

Virus & Rickettsia Division BIOLOGICAL SCIENCES LABORATORIES

Project 1B562602A059

September 1969

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences-National Research Council.

ABSTRACT

The plaque assay procedure recently developed for the typhus and spotted fever groups of rickettsiae is also successful with scrub typhus rickettsiae.

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A PLAQUE ASSAY FOR RICKETTSIA TSUTSUGAMUSHI*

Recent reports^{1,2} have demonstrated the success of a plaque technique using several rickettsiae from the spotted fever and typhus groups. The experiments reported in this paper demonstrate the applicability of the technique to strains of scrub typhus, <u>Rickettsia tsutsugamushi</u>.

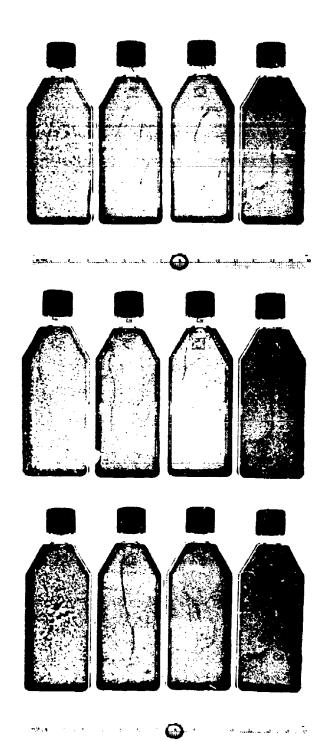
Several vials each of the Karp, Gilliam, and Kato strains of Rickettsia tsutsugamushi (20% yolk sac suspensions in SP-G buffer³) were generously supplied by Dr. Bennett L. Elisberg, Walter Reed Army Institute of Research, Washington, D.C. These strains were in their 47th, 136th, and 89th yolk sac passages, respectively. To expand the volume of working seed material, one vial of each strain was thawed, diluted teniold in sucrose phosphate (SP 25) buffer, dispensed in ampoules, and stored at -65 C. This diluted and refrozen material was used exclusively for plaquing experiments and was not used in any animal tests.

For the plaque assay, 24-hour chick embryo primary monolayers were infected with serial tenfold dilutions, prepared in brain heart infusion broth, of the three strains of R. tsutsugamushi. The infected monolayers were overlayed with 5 ml of medium 199 (5% calf serum) containing 0.5% agarose and incubated at 32 C for 10 days. Three milliliters of a second overlay were placed over the initial overlay after 10 days, and incubation at 32 C was continued for an additional 7 days. Plaques were stained with an overlay containing neutral red as described previously.

The plaque morphology of the three strains of R. tsutsugamushi is shown in Figure 1. The plaques formed by the scrub typhus rickettsiae (1 to 2 mm) are quite similar morphologically to the typhus group plaques shown earlier. However, the scrub typhus organism requires a far longer incubation period (17 versus 10 days) than other typhus-group organisms before plaques appear.

The plaque titer was compared with the animal 50% lethal dose (LD₅₀) and 50% infectious dose (ID₅₀) values. LD₅₀ were determined by injecting groups of ten 16- to 18-g male Swiss mice (Fort Detrick strain) with 0.2 ml of serial tenfold dilutions, prepared in SP 25 buffer, of the original seed materials. Deaths were recorded daily for 25 days. ID₅₀ determinations were made by challenging the survivors on the 26th day with a 1,090 LD₅₀ dose (0.2 ml) of the Karp strain; survivors were presumed to have been previously infected by the initial dose of inoculum. The LD₅₀ and ID₅₀ values were calculated by the Reed-Muench method.

^{*} This report should not be used as a literature citation in material to be published in the open literature. Readers interested in referencing the information contained herein should contact the senior author to ascertain when and where it may appear in citable form.



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FIGURE 1. Plaques Formed by Various Strains of <u>Rickettsia tsutsugamushi</u>. A. Left to right, 10^{-4} , 10^{-5} , and 10^{-6} dilutions of the Karp strain. B. Left to right, 10^{-4} , 10^{-5} , and 10^{-6} dilutions of the Gilliam strain. C. Left to right, 10^{-3} , 10^{-4} , and 10^{-5} dilutions of the Karo strain. At far right in each photo is an uninfected control.

A comparison of the plaque titers and the mouse LD_{∞} and ID_{50} titers is shown in Table 1. These data show that the plaque titration method is more sensitive than the mouse titration performed in our laboratory. Elisberg* previously obtained ID_{50} values greater than 8.0 log_{10}/ml with the same seed pools of these three strains. However, he employed a different strain of mice in his tests (Charles River ICR certified pathogenfree Swiss mice) that may be more sensitive to these organisms than the strain we used.

TABLE 1. COMPARISON OF PLAQUE TITERS OF SEVERAL STRAINS OF RICKETTSIA TSUTSUGAMUSHI WITH LD₅₀
AND ID₅₀ TITERS IN SWISS MICE

Strain	LD ₅₀ log ₁₀ /ml	1D ₅₀ log ₁₀ /ml	Plaque Titer, log _{lo} /ml	
Karp	5.98	6.03	7.54	
Gilliam	≤5.2	5.7	7.39	
Kato	≤5,2	≤5.26	5.97	

Using this procedure we have thus far^{1,2} been successful in plaquing every species of <u>Rickettsia</u> that we have tested. The results of this study and of previous studies suggest that the plaque assay procedure may be appropriate for plaquing all rickettsiae and should greatly facilitate their study.

^{*} Personal communication.

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